Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

LABORATORY BIOSAFETY AND GOOD LABORATORY PRACTICES

11

Risk comes from not knowing what you're doing.
-Warren Buffett, American business magnate, investor, philanthropist.

CHAPTER OUTLINE

11.1 Introduction 254
11.2 Risk Categories of Microorganisms 255
11.3 Biosafety Levels 256
11.3.1 Physical Containment 257
11.3.2 Biological Containment 263
11.4 Physical and Biological Containment for Research Involving Plants 263
11.5 Physical and Biological Containment for Research Involving Animals 267
11.6 Good Laboratory Practice 270
11.7 Summary 271
References 271

Paul Berg opening a jar under a protective hood.

Photo courtesy: Stanford University Archives.

An Introduction to Ethical, Safety and Intellectual Property Rights Issues in Biotechnology.
DOI: http://dx.doi.org/10.1016/B978-0-12-809231-6.00011-9

11.1 INTRODUCTION

Handling organisms including microorganisms under laboratory conditions is an essential part of biotechnology research and applications. Responsible and safe handling of microorganisms (potentially pathogenic) is necessary to ensure the health of laboratory personnel, the community, and the environment. This chapter reviews practices and protocols that have been established at the international and national level to ensure biosafety in institutions involved in biotechnology research and development.

In the early days of the development of recombinant DNA technology, the consensus in the scientific community was that due to the potential to generate new (potentially harmful) forms of organisms, mere **good microbiological techniques (GMT)** alone would not suffice to ensure safety of workers in this area of research. Consequently, in June 1976, shortly after the Asilomar Conference (see Chapter 9: Ensuring Safety in Biotechnology), the US National Institutes of Health (NIH) brought out the ***NIH Guidelines for Research Involving Recombinant Nucleic Acid Molecules*** (henceforth referred to as "**NIH Guidelines**"). (The guidelines have undergone several revisions, the most recent one being in April 2016.) The guidelines recognize six classes of experiments based on the risk involved in the research, which require sanction from different regulatory bodies (see Chapter 9: Ensuring Safety in Biotechnology, Box 9.2).

Recognizing that biological safety is an important international issue, the United Nations World Health Organization (WHO) in 1983 published the ***Laboratory Biosafety Manual*** (henceforth referred to as "**WHO manual**") establishing basic concepts and practices in safe handling of pathogenic microorganisms. **The document encouraged countries to develop national codes of practice for implementation within their geographic boundaries**. The WHO manual has since been revised twice, in 1993 and again in 2004. The third edition incorporates biosecurity concepts in addition to making specific recommendations for biosafety in handling genetically modified organisms.

Adhering to international standards and incorporating biosafety practices in national policies is important not only to protect plant, animal or human life, and health of its citizens, but also in trade in biotech products. Exporting countries need to demonstrate that the measures it applies to its exports achieve the same level of health protection as in the importing countries in order to avoid barriers in trade. Providing oversight to global rules of trade between nations is the mandate of the World Trade Organization. This organization administers the General Agreement on Tariffs and Trade (GATT) (see Chapter 13: Relevance of Intellectual Property Rights in Biotechnology). Article 20 of the GATT allows governments to regulate trade to address biosafety of their citizens provided they do not discriminate or use this clause to disguise protectionism. Countries therefore have made efforts to develop guidelines and appropriate legal frameworks for biosafety regulation. Although the developed countries (e.g., the United States) and regions (e.g., European Union) as leaders in the development of modern biotechnology started to develop these frameworks in the mid-1970s and early 1980s, the developing nations generally started the development of national biosafety systems more recently. The WHO manual has served as an important resource document and is consequently reflected in national regulatory instruments for ensuring biosafety.

11.2 RISK CATEGORIES OF MICROORGANISMS

In both the NIH guidelines and the WHO manual, **four categories of microorganisms used in laboratory work are recognized**. The basis of the classification is the **risk of infection** to laboratory workers and, in the event of escape from the laboratory, to the community. Assigning a microorganism to a risk category is dependent on an initial risk assessment made by the investigator and is based on current knowledge of the:

1. **Pathogenicity** of the organism (all microorganisms do not cause diseases),
2. **Host range** and **mode of transmission** of the organism,
3. **Local availability of effective measures to prevent a disease outbreak**, and
4. **Local availability of effective treatment**.

The Appendix B of the NIH guidelines, *Classification of Human Etiological Agents on the Basis of Hazard*, as does Table 1 of the WHO manual, recognizes four **Risk Groups** of microorganisms:

- ***Risk Group 1***: Microorganisms **unlikely to cause human or animal diseases** and thus pose little or no risk to individuals and to the community; sometimes designated as **generally regarded as safe** (GRAS) organisms (e.g., asporogenic *Bacillus subtilis* or *Bacillus licheniformis*, the K-12 strain of *Escherichia coli*)
- ***Risk Group 2***: Microorganisms that are pathogenic, but **unlikely to pose a serious hazard** to laboratory workers, livestock, the community, or the environment as effective treatment and preventive measures to limit spread of infection are available. These organisms thus are of moderate risk to the individual and low risk to the community (e.g., bacterial agents—*Aeromonas hydrophila*, *E. coli*, *Klebsiella* spp., *Salmonella* spp.; fungal agents—*Penicillium marneffei*, *Blastomyces dermatitidis*; parasitic agents—*Ascaris* spp., *Trypanosoma* spp.; viruses—adenoviruses, Coronaviruses, Papilloma viruses)
- ***Risk Group 3***: Microorganisms that are **pathogenic and can cause serious human or animal diseases, but are not contagious** or have effective treatment and preventive measures. These organisms pose high risk to the individual, but low risk to the community (e.g., bacterial agents—*Brucella* spp., *Francisella tularensis*, *Rickettsia* spp.; fungal agents—*Coccidiodes immitis, Histoplasma* spp.; Viruses and prions—Togaviruses, Flaviviruses such as the Japanese encephalitis virus, West Nile virus, Pox viruses, prions such as the transmissible spongiform encephalopathies, retroviruses such as human immunodeficiency virus, rhabdovirus)
- ***Risk Group 4***: Microorganisms that usually **cause serious diseases in humans** and animals and can be readily transmitted either directly or indirectly from one to the next individual. **Effective treatment and preventive measures are usually unavailable**. This class of organisms thus poses a **high risk** to individuals and to the community (e.g., viral agents such as the Lassa virus, Ebola virus, Marburg virus, Herpes virus simiae, Kayasanur Forest disease, Central European encephalitis, and as yet unidentified hemorrhagic fever agents).

The NIH guidelines recognize that this classification is **dependent on current knowledge** of pathogenicity, and with the development of better therapeutic and preventive measures, pathogens may be assigned to a lower risk category. Different countries may assign the same organism to

different risk groups, possibly because the same organism is more virulent in certain parts of the world than others depending on climatic conditions and other factors. Also, any strain more virulent than the wild-type parent strain should be assigned to a higher risk group.

11.3 BIOSAFETY LEVELS

Both the NIH guidelines and the WHO manual recommend **four Biosafety levels (BLs) 1 to 4** for handling organisms corresponding to the four risk groups. Implementation of safety procedures in each level relies on:

1. **Standard practices** of GMT
2. **Physical barriers** provided by special procedures, equipment, and laboratory installations commensurate with the estimated biohazard.

Appendix G of the NIH guidelines describes four BLs of ***Physical Containment*** summarized in Table 11.1 for standard laboratory experiments. For large-scale (over 10 L) research or production, physical containment requirements are defined in Appendix K (see Chapter 12: Recombinant DNA Safety Considerations in Large-Scale Applications and Good Manufacturing Practice) (WHO Laboratory Biosafety Manual, 2004). The **BL assigned for specific research work** depends on the **assessed risk group of the organisms** handled as well as **professional judgment** of risk associated with the activity.

Table 11.1 Relation of Risk Groups to Biosafety Levels, Practices, and Equipment

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic—Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic—Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment—Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment—Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, *biological safety cabinet;* GMT, *good microbiological techniques.*
Reprinted with permission from WHO Laboratory Biosafety Manual, 2004. Third edition, retrieved from http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf.

11.3.1 PHYSICAL CONTAINMENT

The first principle of physical containment is **strict adherence to good microbial practices**, hence, **all personnel** directly or indirectly working with recombinant or synthetic nucleic acids **should be trained in GMT**. Appendix G-II of the NIH guidelines describes four levels of physical containment **BL1, BL2, BL3, and BL4**, representing facilities in which experiments ranging from **low to high potential hazard** may be conducted. For each BL, the guidelines specify the following:

- Standard microbiological practices,
- Special practices,
- Containment equipment, and
- Laboratory facilities.

Table 11.2 summarizes the facility requirements at the four BLs (for details, see Box 11.1).

Table 11.2 Summary of Biosafety Level Requirements

	Biosafety Level			
	1	**2**	**3**	**4**
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation				
Inward airflow	No	Desirable	Yes	Yes
Controlled ventilating system	No	Desirable	Yes	Yes
HEPA-filtered air exhaust	No	No	Yes/No[b]	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	—
Anteroom with shower	No	No	Yes/No[c]	No
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave				
On site	No	Desirable	Yes	Yes
In laboratory room	No	No	Desirable	Yes
Double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability[d]	No	No	Desirable	Yes

[a] *Environmental and functional isolation from general traffic.*
[b] *Dependent on location of exhaust.*
[c] *Dependent on agent(s) used in the laboratory.*
[d] *For example, window, closed-circuit television, two-way communication.*
Reprinted with permission from WHO Laboratory Biosafety Manual, 2004. Third edition, retrieved from http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf.

BOX 11.1 PHYSICAL CONTAINMENT FOR STANDARD LABORATORY EXPERIMENTS

Appendix G of the **NIH guidelines** identifies strict adherence to good microbiological practices as being the first principle of containment; hence, all personnel directly or indirectly associated with experiments involving recombinant or synthetic nucleic acid molecules should be trained in good microbiological techniques. The four levels of physical containment Biosafety Levels 1 through 4 as described in Appendix G are summarized below:

Biosafety Level 1:

- *Standard microbiological practices:*
  - Access to the laboratory is limited or restricted at the discretion of the Principal Investigator (PI).
  - Work surfaces are decontaminated once a day, all liquid and solid wastes are decontaminated before disposal.
  - Mouth pipetting is prohibited.
  - Eating, drinking, smoking, or storing food in the refrigerators is prohibited.
  - Procedures are performed carefully to prevent formation of aerosols.
  - Good hygiene including washing hands and wearing protective clothes is encouraged.
- *Special practices:*
  - Contaminated materials to be decontaminated at a site away from the laboratory are transported in durable, leak-proof containers with closed lids.
  - An insect and rodent control program is required.
- *Containment equipment:*
  - Generally, not required for BL1
- *Laboratory facilities:*
  - The laboratory should be designed to be easily cleaned.
  - Benchtops should be resistant to water, acid/alkali/organic solvents and should have sinks for hand-washing.

Biosafety Level 2:

- *Standard microbiological practices:*
  - As described for BL1 and
  - Experiments of lesser biohazard can be conducted concurrently in demarcated areas of the laboratory.
- *Special practices:*
  - As described for BL1 and
  - PI limits access to the laboratory and establishes policies and procedures whereby persons entering the laboratory are aware of the hazard and meet any specific entry requirements (such as immunization).
  - The hazard-warning sign with the universal biosafety symbol (Fig. 11.1) with details of the agent used, contact information of the PI, and any special requirements for entry, are to be posted on the access door.
  - Protective clothing used exclusively in the laboratory is required; gloves are to be used to prevent skin contamination with experimental organisms.
  - Only needle-locking hypodermic syringes are used, placed in puncture-proof containers after use, and decontaminated before disposal.
  - A biosafety manual is prepared and adopted for safety of personnel.
  - Baseline serum samples of all laboratory and at-risk personnel should be collected and stored in accordance with institutional policy.
- *Containment equipment:*
  - Biological safety cabinets (class I or II) (Fig. 11.2) or other appropriate personal protective devices are used.
- *Laboratory facilities:*
  - As described for BL1 and
  - An autoclave required for decontamination.

(*Continued*)

BOX 11.1 (CONTINUED)

FIGURE 11.1

Biohazard warning sign for laboratory doors.

Reprinted with permission from WHO Laboratory Biosafety Manual, 2004. Third edition, retrieved from http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf.

Biosafety Level 3:

- *Standard microbiological practices:*
  - As described for BL2 and
  - Persons below 16 years of age are not permitted entry.
- *Special practices:*
  - As described for BL2 and
  - Laboratory doors are kept close when experiments are in progress.
  - Laboratory clothing that protects street clothes is to be worn in the laboratory, removed when exiting the laboratory, and decontaminated prior to laundry or disposal.
  - Molded surgical masks or respirators are worn in rooms containing experimental animals.
  - If animals housed with conventional caging system, personnel must wear protective devices that includes wrap-around gowns, head covers, gloves, shoe covers, and respirators; personnel shall shower on exit from areas where these devices are required.
  - Alternatively, laboratory animals shall be housed in partial-containment caging systems; no animals other than the experimental animals are allowed.
  - Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.

(*Continued*)

BOX 11.1 (CONTINUED)

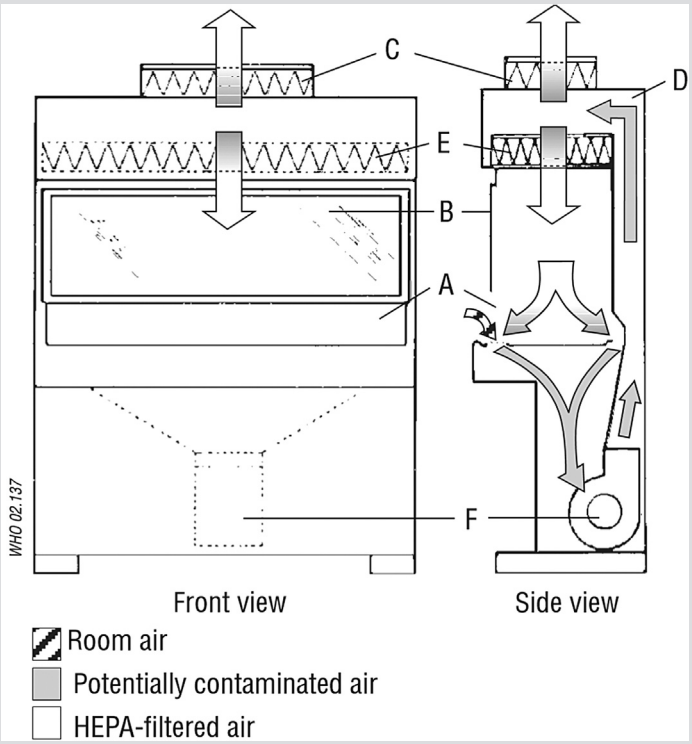

FIGURE 11.2 Schematic representation of a Class II biological safety cabinet.

(A) Front opening; (B) sash; (C) exhaust HEPA filter; (D) rear plenum; (E) supply HEPA filter; (F) blower.

Reprinted with permission from WHO Laboratory Biosafety Manual, 2004. Third edition, retrieved from http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf.

- Spills and accidents which result in potential exposure to modified organisms are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee (IBSC) and to the NIH Office of Science Policy. Written records are to be maintained on appropriate medical evaluation, surveillance, and treatment provided.

- *Containment equipment:*
  - Biological safety cabinets (class I, II, or III) or other appropriate personal protective devices (such as special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, containment cages for animals) are used.
- *Laboratory facilities:*
  - Laboratory to be separated from open areas within the building and accessed through two sets of doors; physical separation of high containment laboratory from other laboratories or activities, may be provided by a double-door clothes change room with showers, airlock, or other double-door access features.
  - Interior surfaces of walls, floors, and ceilings are water resistant for easy cleaning, should be capable of being sealed for decontaminating the area.

(*Continued*)

BOX 11.1 (CONTINUED)

- Access doors are self-closing.
- The HEPA-filtered exhaust air from Class I or II biological cabinets is discharged directly to the outside or through the building exhaust system.

Biosafety Level 4:

- *Standard microbiological practices:*
  - As described for BL3
- *Special practices:*
  - As described for BL3 and
  - Access to the facility is limited by means of secure locked doors; accessibility is restricted to authorized personnel and is supervised and managed by the PI, Biological Safety Officer, or person responsible for the physical security of the facility. A log of entry and exit of personnel is maintained. All personnel are advised of potential biohazards and are to comply with instructions on entry and exit procedure. Protocols for emergency situations are established.
  - Biological material to be removed in an intact state are to be sealed in a primary nonbreakable container, enclosed and sealed in a secondary nonbreakable container, and removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for the purpose.
  - Any other material to be removed from the facility are to be autoclaved or decontaminated before exiting the maximum containment laboratory.
  - Personnel enter and exit the facility only through clothing change and shower rooms; shower every time they exit the facility.
  - Street clothing is removed and kept in an outer changing room. Complete laboratory clothing (may be disposable) is provided and to be used by all personnel entering the facility. When exiting, the laboratory clothing is removed in an inner changing room before proceeding to the shower area. The clothing is decontaminated prior to laundering or disposal.
  - Supplies and material are brought into the facility through a double-door autoclave, fumigation chamber, or airlock.
- *Containment equipment:*
  - All procedures within the maximum containment facility are conducted in the Class III biological safety cabinet (Fig. 11.3), or in a Class I or II biological safety cabinet used in conjunction with a one-piece positive pressure personnel suits ventilated by a life-support system.
- *Laboratory facilities:*
  - The maximum containment facility is to be housed in a separate building or a clearly demarcated and isolated zone within a building. Access to the facility requires outer and inner change rooms separated by showers for entry and exit of personnel, and double-door autoclave, fumigation chamber or airlock for passage of materials, supplies, and equipment.
  - Internal surfaces of walls, floors, and ceilings of the facility should be water, acid, and alkali resistant; the facility should be sealable for fumigation, animal and insect proof. Drains in the floor contain traps filled with suitable chemical disinfectant and are connected directly to the liquid-waste decontamination system. Sewer and other ventilation lines contain HEPA filters.
  - Benchtops have seamless surfaces impervious to acids, alkalis, organic solvents, and moderate heat; construction of the facility should have adequate space for accessibility for cleaning.
  - Access doors are self-closing and locking.
  - An individual supply and exhaust air ventilation system that maintains pressure differentials and directional airflow ensures that airflow inwards from areas outside the facility toward areas of highest potential risk within the facility. The supply and exhaust airflow is monitored by manometers to assure inward (or zero) airflow at all times.

(*Continued*)

BOX 11.1 (CONTINUED)

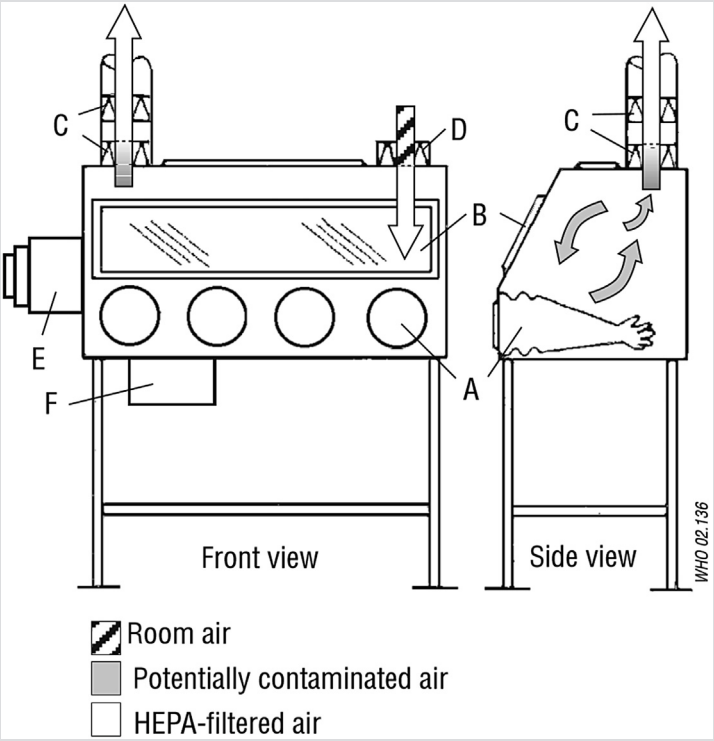

FIGURE 11.3 Schematic representation of a Class III biological safety cabinet (glove box).

(A) glove ports for arm-length gloves; (B) sash; (C) double-exhaust HEPA filters; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box; (F) chemical dunk tank. Connection of the cabinet exhaust to an independent building exhaust air system is required.

Reprinted with permission from WHO Laboratory Biosafety Manual, 2004. Third edition, retrieved from http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf.

- Exhaust air from the facility is filtered through HEPA filters before discharge to the outside.
- A specially designed suit area may be provided in the facility entry into which is through an airlock fitted with airtight doors. The air pressure within the suit area is maintained greater than that of adjacent areas. Personnel who enter this area shall wear a one-piece positive pressure suit ventilated by life-support system. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area.

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) retrieved from http://osp.od.nih.gov/sites/default/files/NIH_Guidelines_0.pdf.

11.3.2 BIOLOGICAL CONTAINMENT

The growth and dissemination of organisms are naturally limited. For ensuring safety, biological containment takes advantage of these natural barriers such as:

1. The infectivity or **host specificity** of a vector or virus
2. Its **spread and survival** in the environment.

Appendix I of the NIH guidelines describes ***Biological containment*** strategies for recombinant or synthetic nucleic acid molecules. The ***vector*** (plasmid, organelle, or virus) for the recombinant or synthetic nucleic acid molecule and ***the host*** (bacterial, animal, or plant cell), in which the vector is propagated, are taken together as a ***Host−Vector system*** for consideration of biological containment. Selection of a Host−Vector system aims to minimize:

1. Survival of the vector in its host outside the laboratory and
2. Transmission of the vector from the propagation host to other nonlaboratory hosts.

Host−Vector 1 Systems provide moderate level of containment. The **EK1 system** has ***E. coli* K-12 (or derivatives) as the host**, and the vectors include **nonconjugative plasmids** (e.g., pSC101, Co1E1) and **variants of bacteriophage lambda**.

Host−Vector 2 Systems (EK2) provide a **high level of biological containment** with escape of the recombinant or synthetic nucleic acid molecule to other organisms under specified conditions being $< 1/10^8$.

11.4 PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RESEARCH INVOLVING PLANTS

The BLs 1 through 4 are applicable to microorganisms, but Appendix P of the NIH guidelines specifies physical and biological containment conditions for experiments involving **recombinant or synthetic nucleic acids in plants, plant-associated microorganisms, and small animals**. The plants include, but are not limited to, mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species. Plant-associated microorganisms include those that have a benign or beneficial effect as also those that cause diseases, and include viroids, virusoids, viruses, bacteria, fungi, protozoans, small algae, as well as microbes being modified for association to plants. Plant-associated small animals include arthropods and nematodes, tests on which require the use of plants. **The purpose of the containment is to prevent unintentional transmission of recombinant or synthetic nucleic acid molecule containing plant genomes (nuclear or organellar DNA), or release of modified organisms associated with plants**. Appendix P-II establishes four levels referred to as BL1-Plants (P), BL2-P, BL3-P, and BL4-P, which specify the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Appendix P-III specifies Biological Containment Practices if botanical reproductive structures are produced that can potentially be released. For further details, see Box 11.2.

BOX 11.2 PHYSICAL CONTAINMENT FOR EXPERIMENTS INVOLVING PLANTS

Appendix P of the **NIH guidelines** supersedes Appendix G (Physical Containment) when the research plants are of a size, number, or have growth requirements that preclude use of the containment conditions outlined in Appendix G. The containment principles in Appendix P are based on the premise that the organisms pose no health threat to humans or higher animals and that the purpose of the containment is to minimize the possibility of unanticipated deleterious effects on organisms and the ecosystem. The physical containment levels describe greenhouse practices and special greenhouse facilities for physical containment.

Biosafety Level 1—Plants (BL1-P):

- *Standard practices:*
  - *Greenhouse access:*
    - Limited or restricted, at the discretion of the Greenhouse director when experiments are in progress.
    - Personnel shall be required to follow standard greenhouse procedures.
  - *Records:*
    - Record shall be maintained of experiments in progress in the facility.
  - *Decontamination and inactivation:*
    - Experimental organisms shall be rendered biologically inactive by appropriate methods prior to disposal.
  - *Control of undesired species and motile macroorganisms:*
    - Appropriate methods shall be adopted to control undesired species of weeds, rodents, arthropod pests, and pathogens.
    - If macroorganisms are released in the greenhouse, precautions are to be taken to minimize escape from the greenhouse facility.
  - *Concurrent experiments conducted in the greenhouse:*
    - Provided the work is conducted in accordance with BL1-P practices, experiments involving other organisms that require containment level lower than BL1-P may be conducted.
- *Greenhouse design:*
  - The floor of the greenhouse may be of gravel or other porous materials, impervious (concrete) walkways are recommended.
  - The greenhouse may be vented with windows or other openings in walls or roof, screens are recommended as barriers to contain or exclude pollen, microorganisms, or small flying animals.

Biosafety Level 2—Plants (BL2-P):

- *Standard practices:*
  - *Greenhouse access:*
    - As described for BL1-P
    - Personnel should be aware of and follow BL2-P practices and procedures.
  - *Records:*
    - As described for BL1-P and
    - The PI shall report any greenhouse accident involving inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, the NIH Office of Science Policy, and other appropriate authorities. Written records are to be maintained on any such accident.
  - *Decontamination and inactivation:*
    - As described for BL1-P and
    - Decontamination of run-off water is not generally required, although periodic cleaning to remove any organisms potentially entrapped by the gravel is to be done.
  - *Control of undesired species and motile microorganisms:*
    - As described for BL1-P
  - *Concurrent experiments conducted in the greenhouse:*
    - As described for BL1-P

(*Continued*)

BOX 11.2 (CONTINUED)

- *Signs:*
  - A sign shall be posted to indicate that a restricted experiment is in progress, shall indicate the name of the responsible person, the plants in use, and any special requirements for using the area.
- *Transfer of materials:*
  - Materials containing experimental organisms brought into or removed from the greenhouse facility in a viable state shall be transferred in a closed nonbreakable container.

- *Greenhouse design:*
  - As described for BL1-P.
  - An autoclave shall be available for treatment of contaminated greenhouse materials.

Biosafety Level 3—Plants (BL3-P):

- *Standard practices:*
  - *Greenhouse access:*
    - As described for BL1-P.
    - Personnel should be aware of and follow BL3-P practices and procedures.
  - *Records:*
    - As described for BL2-P.
  - *Decontamination and inactivation:*
    - All experimental materials including water shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal (except those that are to remain in a viable or intact state for experimental purposes).
  - *Control of undesired species and motile microorganisms:*
    - As described for BL1-P.
    - Arthropods and other motile macroorganisms shall be housed in appropriate cages; when appropriate to the organism, experiments shall be conducted in the cages.
  - *Concurrent experiments conducted in the greenhouse:*
    - Involving organisms that require containment lower than BL3-P may be conducted concurrently provided BL3-P practices are followed.
  - *Signs:*
    - As described for BL2-P and
    - If organisms used have a recognized potential for causing detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access door.
    - If there is a risk to human health, a sign with the universal biosafety symbol shall be posted.
  - *Transfer of materials:*
    - A sealed nonbreakable secondary container shall be used for experimental material brought into or removed from the greenhouse facility in a viable state.
    - At the time of transfer, the surface of the secondary container shall be decontaminated by passage through a chemical disinfectant or fumigation chamber or any method found effective.
  - *Protective clothing:*
    - Disposable clothing (such as solid front or wrap around gowns, scrub suits, or other appropriate clothing) shall be worn if deemed necessary by the Greenhouse Director.
    - Such clothing shall be removed before exiting the facility and decontaminated before laundering or disposal.
- *Greenhouse design:*
  - The greenhouse floor shall be of concrete or other impervious material with provision to collect and decontaminate liquid run-off.
  - Windows shall be sealable, glazing shall be resistant to breakage; internal walls, ceilings, and floors shall be resistant to penetration by liquids to facilitate cleaning and decontamination; benchtops and other surfaces should be seamless, resistant to acids, alkali, organic solvents, and moderate heat; a foot, elbow, or automatically operated sink should be located near the exit for hand washing.

(*Continued*)

BOX 11.2 (CONTINUED)

- The greenhouse shall be a closed self-contained structure, separated from areas open to unrestricted flow of traffic; it shall be surrounded by a security fence or protected by security measures.
- An autoclave (double door recommended) shall be available for decontaminating materials within the facility.
- An individual supply and exhaust air ventilation shall be provided that maintains pressure differentials and directional airflow (assures inward, or zero, airflow from areas outside the greenhouse).
- Exhaust air shall be filtered through HEPA filters prior to discharge.

Biosafety Level 4—Plants (BL4-P):

- *Standard practices:*
  - *Greenhouse access:*
    - As described for BL3-P and
    - Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the facility; airlocks are used only for emergency exits; all reasonable efforts taken to ensure that viable propagules are not transported from the facility in an emergency.
    - Prior to entry, personnel should read and follow instructions on BL4-P procedures.
  - *Records:*
    - As described for BL2-P and
    - A record and time-log is kept of all people entering or exiting the facility.
  - *Decontamination and inactivation:*
    - As described for BL3-P and
    - Water that comes in contact with the experimental material (such as run-off water) shall be collected and decontaminated before disposal; all equipment and materials used will be decontaminated as in standard microbiological practices.
  - *Control of undesired species and motile microorganisms:*
    - As described for BL3-P
  - *Concurrent experiments conducted in the greenhouse:*
    - Experiments involving organisms that require containment less than BL4-P may be conducted concurrently.
  - *Signs:*
    - As described for BL3-P
  - *Transfer of material:*
    - As described for BL3-P and
    - Supplies and materials shall be brought into the facility through a double-door autoclave, fumigation chamber, or airlock that is fumigated between uses.
  - *Protective clothing:*
    - Street clothing is removed and kept in an outer changing room. Complete laboratory clothing (may be disposable) is provided and to be used by all personnel entering the facility. When exiting, the laboratory clothing is removed in an inner changing room before proceeding to the shower area. The clothing is decontaminated prior to laundering or disposal.
- *Greenhouse design:*
  - The maximum containment greenhouse shall consist of a separate building or a clearly demarcated area; should be able to maintain negative pressure; surrounded by a security fence or similar security measures.
  - Outer and inner change rooms separated by a shower shall be provided for entry and exit of personnel; doors should be self-closing; windows closed and sealed; glazing shall be resistant to breakage; ceilings and floors shall be resistant to penetration by liquids to facilitate cleaning and decontamination; benchtops and other surfaces should be seamless, resistant to acids, alkali, organic solvents, and moderate heat.
  - A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of materials, supplies, and equipment.
  - An individual supply and exhaust air ventilation shall be provided that maintains pressure differentials and directional airflow (assures inward, or zero, airflow from areas outside the greenhouse).
  - Exhaust air shall be filtered through HEPA filters prior to discharge.

11.5 PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RESEARCH INVOLVING ANIMALS

Appendix Q of the NIH guidelines deals with the requirements for containment and confinement for research involving whole animals. The guideline covers both animals whose genome has been altered by stable integration of recombinant or synthetic nucleic acid molecules into the germ line (**transgenic animals**), as well as **modified microorganisms tested on whole animals**. The animals covered in the guidelines include, but are not limited to, cattle, swine, goats, horses, sheep, and poultry. As in the case of plants, four levels of containment are established, referred to as BL1- Animals (N), BL2-N, Bl3-N, and BL4-N. For further details, see Box 11.3.

BOX 11.3 PHYSICAL CONTAINMENT FOR EXPERIMENTS INVOLVING ANIMALS

Appendix Q of the **NIH guidelines** supersedes Appendix G (Physical Containment) when the animals are of a size or have growth requirements that preclude the use of the physical containment described in Appendix G. For experiments that require prior approval of the IBSC that utilize facilities described in Appendix Q, the IBSC shall include at least one scientist with expertise in animal containment principles. The institute shall establish a health surveillance program for personnel working with viable microorganisms carrying recombinant or synthetic DNA that require BL 3 or greater.

Biosafety Level 1—Animals (BL1-N):

- *Standard practices:*
  - *Animal facility access:*
    - The containment area shall be locked; access shall be limited or restricted when experiments are in progress; the area shall be patrolled/monitored at frequent intervals.
    - The containment area shall be in accordance with state and Federal laws and animal care requirements.
    - All genetically engineered neonates shall be permanently marked within 72 hours of birth (or if size does not permit, the containers shall be marked); transgenic animals should contain distinct and biochemically assayable DNA sequences that allow distinction between modified and nonmodified animals; a double barrier shall separate male and female animals unless reproductive studies are part of the study.
- *Animal facilities:*
  - Animals shall be confined to securely fenced areas or enclosed animal rooms to minimize the possibility of theft or unintended release.

Biosafety Level 2—Animals (BL2-N):

- *Standard practices:*
  - *Animal facility access:*
    - As described for BL1-N and
    - The Animal Facility Director shall establish procedures to ensure personnel who enter are advised of potential hazards and meet specific requirements (such as vaccination); animals of the same or different species, not involved in the experiment, shall not be permitted.
  - *Decontamination and inactivation:*
    - Materials to be decontaminated elsewhere are to be placed in closed durable leak-proof containers, needles and syringes in puncture-proof containers to be autoclaved before disposal.
  - *Signs:*
    - Warning signs incorporating the universal biosafety symbol to be posted on access doors containing details of special provisions (such as vaccinations) for entry, agents and animal species involved in the experiments, and details of the Animal Facility Director.

(*Continued*)

BOX 11.3 (CONTINUED)

- *Protective clothing:*
  - Protective coating to be worn in the animal area, to be removed in nonlaboratory areas, gloves to be worn and care to be taken to avoid skin contamination.
- *Records:*
  - Any incident involving spills or inadvertent exposure or release of modified microorganisms shall be reported to the Animal Facility Director, Institutional Biosafety Committee, the NIH Office of Science Policy, and other appropriate authorities. Written records are to be maintained on any such accident, and if necessary, the area shall be decontaminated.
  - When appropriate, base line serum samples of animal care workers and at-risk personnel may be collected and stored.
- *Transfer of materials:*
  - Advance approval for transfer of material shall be obtained from the Animal Facility Director; biological material shall be transferred in a sealed nonbreakable primary container, sealed in a second nonbreakable container, both of which are to be disinfected before removal; unless inactivated, packages are to be opened in a facility having equivalent or higher physical containment.
- *Other:*
  - As described for BL1-N and
  - Appropriate steps to be taken to prevent horizontal transmission or exposure of personnel; eating, drinking, smoking is not permitted in the work area.

- *Animal facilities:*
  - As described for BL1-N and
  - Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, moderate heat, easy to clean; windows that open shall be fitted with fly screens; special attention to be taken to prevent entry and exit of arthropods.

Biosafety Level 3—Animals (BL3-N):

- *Standard practices:*
  - *Animal facility access:*
    - As described for BL2-N
  - *Decontamination and inactivation:*
    - As described for BL2-N and
    - Special safety testing, decontamination procedures, and IBSC approval require for transfer of agents or tissue/organ specimens from a BL3-N to a facility of lower containment classification.
    - Liquid effluent from the facility shall be decontaminated by heat treatment prior to release to the sanitary system.
  - *Signs:*
    - As described for BL2-N
  - *Protective clothing:*
    - Full protective clothing shall be worn in the animal area; personnel are required to shower before exiting the BL3-N facility; protective clothing shall not be worn outside the containment area and will be decontaminated before laundering or disposal.
    - Appropriate respiratory protection shall be worn in the containment rooms.
  - *Records:*
    - As described for BL2-N and
    - A permanent record book shall maintain details of experimental animal use and disposal.
  - *Transfer of materials:*
    - As described for BL2-N and
    - Special safety testing, decontamination procedures, and IBSC approval require for transfer of agents or tissue/organ specimens from a BL3-N to a facility of lower containment classification.
  - *Other:*
    - As described for BL2-N

(*Continued*)

BOX 11.3 (CONTINUED)

- *Animal facilities:*
  - As described for BL2-N and
  - The animal containment area shall be separated from other areas; access doors shall be self-closing; passage through two sets of doors and clothes change room equipped with integral showers and airlock.
  - An exhaust air ventilation system shall be provided that creates a directional airflow; that draws air into the animal rooms vacuum lines shall be protected with HEPA filters; liquid effluent from containment rooms shall be decontaminated before discharge into the sanitary system.

Biosafety Level 4—Animals (BL4-N):

- *Standard practices:*
  - *Animal facility access:*
    - As described for BL3-N and
    - Individuals below 16 years of age shall not be permitted to enter the animal area.
    - Personnel shall enter and exit through the clothing change and shower rooms, and use the airlocks in case of an emergency.
  - *Decontamination and inactivation:*
    - As described for BL3-N and
    - All contaminated liquid and solid wastes and wastes from the animal rooms shall be decontaminated before disposal.
  - *Signs:*
    - As described for BL3-N
  - *Protective clothing:*
    - Street clothes shall be removed and kept in the outer changing room; complete laboratory clothing (may be disposable) shall be provided for all personnel entering the animal facility, which is to be removed and placed in bins in the inner changing room while exiting the facility; clothing is decontaminated before laundering or disposal; personnel shall shower each time they exit the containment facility.
    - A ventilated head-hood or a one-piece positive pressure suit shall be worn by personnel entering rooms that contain experimental animals when appropriate.
  - *Records:*
    - As described for BL3-N and
    - A permanent record and time-log of entry and exit of personnel is to be maintained.
  - *Transfer of materials:*
    - As described for BL3-N and
    - Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock appropriately decontaminated between use.
  - *Other:*
    - As described for BL3-N and
    - Animal-holding areas shall be cleaned at least once a day and decontaminated immediately if spilling of viable materials occurs.
    - An essential adjunct to the reporting, surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated diseases.
- *Animal facilities:*
  - As described for BL3-N and
  - The BL4-N shall have a double barrier to prevent release of recombinant or synthetic nucleic acid molecule containing microorganisms to the environment such that even if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment; physical separation of the animal containment area is by double-door clothes change room equipped with showers and airlock.
  - All equipment and floor drains shall be equipped with minimally 5-in.-deep traps; ducted exhaust air ventilation shall be provided that is filtered through double HEPA filters and creates a directional airflow that draws air into the laboratory.

11.6 GOOD LABORATORY PRACTICE

By definition "*Good Laboratory Practice embodies a set of principles that provide a framework within which laboratory studies are planned, performed, monitored, recorded, reported, and archived*" (Dolan, 2007). The primary purpose of GLP is to **ensure uniformity, consistency, and reliability of safety tests (nonclinical) for pharmaceuticals, agrochemicals, aroma and color food/feed additives, cosmetics, detergents, novel foods, nutritional supplements for livestock, and other chemicals**. These safety tests are used to generate data on various parameters from physicochemical properties to toxicity (nonclinical) for use of regulatory authorities in order to make risk/safety assessments. Originally, GLP regulations were intended for toxicity testing only and were reserved for laboratories undertaking animal studies for preclinical work. **GLP is now followed in all laboratories where research or marketing studies are to be submitted to regulatory authorities** such as the FDA. **Establishment of GLP is mandatory to evaluate safety or toxicity of products intended to undergo clinical trials.**

Historically, GLP was introduced in several countries (including the United States in 1978) in response to a scandal involving an American industrial product safety testing laboratory in Illinois, the Industrial Bio-Test (IBT) Laboratory. This laboratory performed more than one-third of all toxicology testing in the United States in the 1950s to 1970s, but was found guilty of extensive scientific misconduct, resulting in indictment and convictions of several of its staff in the early 1980s. As data generated by IBT had been used by regulatory authorities for marketing licenses, the United States Environmental Protection Agency was forced to pull several pesticides from the market pending reevaluation of its safety data.

The **Organization for Economic Cooperation and Development *Principles of Good Laboratory Practice (GLP)*** was first developed in 1978 by an Expert Group led by the United States with experts from Australia, Austria, Belgium, Canada, Denmark, France, the Federal Republic of Germany, Greece, Italy, Japan, the Netherlands, New Zealand, Norway, Sweden, Switzerland, the United Kingdom, the Commission of the European Communities, the WHO, and the International Organization for Standardization. The GLP was formally recommended for use in Member countries in 1981. A more comprehensive document specifying the Principles of GLP was brought out by the OECD in 1992 (revised in 1997) (OECD, 1998) and has since been adopted by several countries and incorporated in national regulatory policies and documents.

Compliance with GLP requires that:

1. The tests should be conducted by **qualified personnel**.
2. Each study should have a **Study Director responsible** for the overall conduct of the tests.
3. The laboratory study and the accompanying data should be **audited by a Quality Assurance Unit.**
4. All laboratory activities must be performed in accordance with **written and filed management-approved Standard Operating Procedures (SOPs)**. SOPs should cover policies, administration, equipment operation, technical operation, and analytical methods.
5. All control and test articles and reagents must be **identified, characterized, and labeled** with information regarding **source, purity, stability, concentration, storage conditions, and expiration date**.
6. The equipment must be **maintained, calibrated, and must be designed to meet analytical requirements**.

Compliance with GLP has served to harmonize test methods across nations, facilitating generation of **mutually acceptable data**, thus avoiding duplication of tests, and saving time and resources.

KEY TAKEAWAYS

The primary purpose of **GLP** is to ensure uniformity, consistency, and reliability of safety tests (nonclinical) for pharmaceuticals, agrochemicals, aroma and color food/feed additives, cosmetics, detergents, novel foods, nutritional supplements for livestock, and other chemicals. **Establishment of GLP is mandatory to evaluate safety or toxicity of products intended to undergo clinical trials**.

11.7 SUMMARY

Crucial to the research and development of new applications of genetically modified organisms derived by the transfer of synthetic or recombinant nucleic acid molecules are measures to prevent hazards (to laboratory personnel as well as to other persons, animals, and the ecosystems) from being realized. Guidelines prepared by the NIH and the WHO have helped establish processes and systems that build on GMT in order to ensure biosafety. The guidelines form an integral part of normative policies and regulation of genetically modified organisms in countries using recombinant DNA technology. Both the NIH and the WHO guidelines recommend classification of biological agents based on their potential to cause harm to humans, animals, and the environment. Four BLs are recommended to handle organisms of increasing risk potential. Recommended for each level are standard microbiological practices as well as facilities for physical and biological containment of genetically modified organisms (microbes, plants, or animals). In order to harmonize toxicity testing and generation of mutually acceptable preclinical data that may be used for decisions regarding regulation including commercialization, several countries have adopted the principles of GLPs. These principles establish a framework and a minimum standard for the conduct of tests, and documentation and analysis of data.

REFERENCES

Dolan, K. (2007). *Laboratory animal law: Legal control of the use of animals in research*. Oxford: Blackwell Publishing Limited.

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) retrieved from http://osp.od.nih.gov/sites/default/files/NIH_Guidelines_0.pdf.

OECD (1998). *OECD principles on good laboratory practice (as revised in 1997). OECD series on principles of good laboratory practice and compliance monitoring number 1*. Paris: Environment Directorate, Organisation for Economic Co-operation and Development. Retrieved from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en.

WHO Laboratory Biosafety Manual 2004 Third edition, retrieved from http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf.